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Investigation

Suppression of meiotic crossovers in pericentromeric heterochromatin requires synaptonemal complex and meiotic recombination factors in *Drosophila melanogaster*

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The centromere effect (CE) is a meiotic phenomenon that ensures meiotic crossover suppression in pericentromeric regions. Despite being a critical safeguard against nondisjunction, the mechanisms behind the CE remain unknown. Previous studies found that different regions of the *Drosophila* pericentromere, encompassing proximal euchromatin, beta, and alpha heterochromatin, undergo varying levels of crossover suppression, raising the question of whether distinct mechanisms establish the CE in different regions. We asked whether different pericentromeric regions respond differently to mutations that impair features that may play a role in the CE. In flies with a mutation that affects the synaptonemal complex (SC), a structure that is hypothesized to have roles in recombination and crossover patterning, we observed a redistribution of pericentromeric crossovers from proximal euchromatin towards beta heterochromatin but not alpha heterochromatin, indicating a role for the SC in suppressing crossovers in beta heterochromatin. In flies mutant for *mei-218* or *rec*, which encode components of a critical pro-crossover complex, there was a more extreme redistribution of pericentromeric crossovers towards both beta and alpha heterochromatin, suggesting an important role for these meiotic recombination factors in suppressing heterochromatic crossovers. We mapped crossovers in flies mutant for *Su(var)3-9*, which encodes histone H3-lysine-9 methyltransferase. Although we expected strong alleviation of crossover suppression in heterochromatin, no changes in pericentromeric crossover distribution were observed in this mutant, indicating that this vital heterochromatin factor is dispensable for preventing crossovers in heterochromatin. Thus, in *Drosophila. melanogaster* the meiotic machinery seems to play a more significant role in suppressing centromere-proximal crossovers than chromatin state.

Keywords: meiotic crossovers; centromere effect; *Drosophila*; heterochromatin; FlyBase

Introduction

During the first meiotic division, recombination between homologous chromosomes is a crucial process that is required to promote their accurate segregation away from one another—reviewed in Koehler *et al.* (1996). Meiotic crossovers are highly regulated, with the meiotic cell tightly governing where along each chromosome crossovers can form. The rules that control crossover placement are commonly referred to as crossover patterning events (reviewed in Pazhayam *et al.* 2021) and are an additional requirement in ensuring that homologs disjoin correctly during meiosis.

The meiotic crossover patterning features that have been described (Sturtevant 1913; Beadle 1932; Owen 1950; Martini *et al.* 2006), including the exclusion of crossovers near the centromere—commonly referred to as the centromere effect (CE)—occur animals, fungi, and plants (Mahtani and Willard 1998; Copenhaver *et al.* 1999; Wu *et al.* 2003; Ghaffari *et al.* 2013; Vincenten *et al.* 2015; Nambiar and Smith 2016; Fernandes *et al.* 2024). *Drosophila* and human research has shown a correlation between centromere-proximal crossovers and nondisjunction (Koehler *et al.* 1996; Lamb *et al.* 1996; Oliver *et al.* 2012).

Despite the importance of the CE in protecting against meiotic NDJ, little is known about how the CE is established or maintained. Studies of the CE over the past century have largely been split on crossover suppression in centromere-proximal euchromatin having influences from adjacent heterochromatin/repetitive DNA (Slatis 1955; John 1985; Westphal and Reuter 2002) or being entirely dependent on distance from the centromere (Mather 1939; Yamamoto and Miklos 1978). Based on the range of conclusions presented in these studies, it seems highly likely that neither the centromere nor heterochromatin are the final arbiters of centromere-proximal crossover suppression in *Drosophila*. Whether the CE is controlled by one primary mechanism of action, or several factors that must act together to suppress recombination in the region remains an unanswered question in the field, as does the identity and nature of these factors. Although the CE has largely remained a mechanistic mystery since its discovery, certain modes of control have been ruled out in *Drosophila melanogaster*. Disruption of centromere clustering, changes in centromere number, and changes in repetitive DNA

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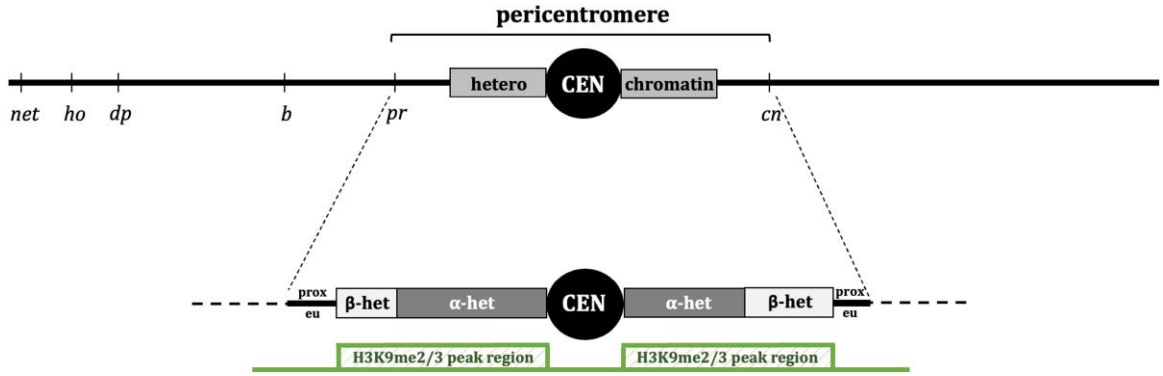


Fig. 1. Schematic of chromosome 2 in *D. melanogaster*. Grey boxes indicate pericentromeric heterochromatin, thick black lines indicate euchromatin along which phenotypic markers used to map recombination are shown. Centromere is indicated as CEN. In the lower image, alpha heterochromatin is indicated as α-het, beta heterochromatin as β-het, and proximal euchromatin as prox-eu and are shown to scale. The size of alpha heterochromatin shown here assumes that all remaining unassembled sequence is alpha heterochromatin. Dashed lines indicate euchromatin that is not considered centromere-proximal and therefore excluded from our definition of the pericentromere. Regions of the chromosome marked by H3K9me2/3 [data from various studies summarized in Stutzman *et al.* (2024)] are shown as green boxes below the lower image.

dosage were shown to have no *trans*-acting effects on the strength of the CE (Pazhayam *et al.* 2024).

The pericentromeric region in *D. melanogaster*, as well as many other organisms, including mammals, *Arabidopsis*, and fission yeast, consists of a centromere embedded in large chunks of heterochromatinized repetitive DNA (Miklos and Cotsell 1990; Simon *et al.* 2015; Ghimire *et al.* 2024). Pericentromeric heterochromatin in *Drosophila* is heterogeneous (Fig. 1), comprising 2 classes defined by sequence, staining patterns, and replication status. This is most clearly seen in polytene chromosomes, where the centromeres are embedded in regions that are densely staining and highly under-replicated, and the adjacent regions are more diffusely stained and are less under-replicated (Gall *et al.* 1971; Miklos and Cotsell 1990). The former, referred to as alpha heterochromatin, is composed largely of tandem arrays of highly repetitive satellite DNA sequences. The moderately stained regions, referred to as beta heterochromatin, are found between alpha heterochromatin and euchromatin, and have a high density of transposable elements interspersed within unique sequence. The unique sequences found in beta heterochromatin have made it possible to assemble much of it to the reference genome (Hoskins *et al.* 2015), whereas the alpha heterochromatin has not yet been assembled.

These 2 classes of centromere-proximal heterochromatin also differ in crossover-suppression patterns. Hartmann et al. showed that in wild-type flies, meiotic crossovers are completely absent from alpha-heterochromatic regions, whereas crossover frequencies in beta heterochromatin and proximal euchromatin depend on distance from the centromere (Hartmann *et al.* 2019b). A similar pattern of centromere-proximal crossover suppression has been described in *Arabidopsis thaliana* (Fernandes *et al.* 2024), where the pericentromere is organized similarly to that of *D. melanogaster*, with the centromere embedded in regions of highly repetitive heterochromatinized DNA that give way to less repetitive heterochromatinized DNA, followed by unique euchromatic sequence. As in *Drosophila*, crossovers in *Arabidopsis* are also completely suppressed in highly repetitive DNA and dependent on distance from the centromere in less repetitive DNA (Fernandes *et al.* 2024).

The existence of these 2 components of the CE raises the question of how they are established during meiosis, and whether distinct processes are responsible for their establishment and execution. It has been previously speculated that the "controlling systems" preventing crossovers in centromere-proximal euchromatin are different from those that prevent crossovers in pericentromeric heterochromatin (Carpenter and Baker 1982; Szauter 1984), leading us to attempt to tease apart the mechanistic differences in proximal crossover suppression within the various regions of the pericentromere, including any—if they exist—between alpha and beta heterochromatin.

Evidence for centromere-proximal crossover suppression being a meiotically controlled phenomenon is abundant. Since the meiotic program has many moving parts, we focused on 2 facets: the synaptonemal complex (SC) and the proteins directing meiotic recombination. The SC is a protein structure that forms during meiosis between paired homologs and is the context within which meiotic recombination occurs. SC has been shown to be necessary for crossover formation as well as patterning in many species (Sym and Roeder 1994; Storlazzi *et al.* 1996; Page and Hawley 2001; Libuda *et al.* 2013; Voelkel-Meiman *et al.* 2015, 2016; Wang *et al.* 2015; Billmyre *et al.* 2019). It has been proposed that the SC has liquid crystalline properties that helps mediate crossover designation and interference by providing a compartment within with the proteins that carry out these processes can diffuse (Rog *et al.* 2017; Morgan *et al.* 2021; Zhang *et al.* 2021; von Diezmann *et al.* 2024). SC in pericentromeric heterochromatin has been reported to have morphological differences from the SC along euchromatin (Carpenter 1975). Compared with euchromatic SC, heterochromatic SC has a more amorphous central element, is less thick, and not as rigid. Heterochromatic SC is also surrounded by chromatin in a way that euchromatic SC is not. A 2019 study showed that the *Drosophila* SC component C(3)G plays a definitive role in suppressing pericentromeric crossovers (Billmyre *et al.* 2019). Collectively, these observations suggest the SC may have a crucial role in establishing the CE.

The second facet is the proteins that direct meiotic recombination. Hatkevich *et al.* (2017) showed that loss of Bloom syndrome helicase, an important DNA repair protein, lacked not only the CE, but also other forms of crossover patterning, such as interference (Hatkevich *et al.* 2017). A 2018 study showed that the introduction of *D. mauritiana* orthologs of the pro-crossover genes *mei-217* and *mei-218* into *D. melanogaster mei-218* mutants attenuated crossover suppression around the centromere, as it is in *D. mauritiana*, suggesting that these genes mediate the strength of the CE in *D. melanogaster* (Brand *et al.* 2018). Mei-217 and Mei-218 are components of the meiotic-mini-chromosome-maintenance (mei-MCM)

complex that is hypothesized the block the anti-crossover activity of Blm (Kohl *et al.* 2012). Analysis of the data of Hartmann *et al.* (2019) suggests that both *mei-218* and *rec*, which encodes the third component of the mei-MCM complex, may contribute to crossover suppression around the centromere. This, and data from other organisms showing genetic modes of suppressing pericentromeric crossovers through blocking or preventing Spo11-mediated meiotic DSBs (Vincenten *et al.* 2015; Nambiar and Smith 2018; Xue *et al.* 2018), suggests that the meiotic program is able to exert considerable control over the CE.

The heterochromatic nature of the pericentromere could also be a key factor contributing to the CE. Crossover suppression within heterochromatin as well as an effect of heterochromatin on crossover suppression in adjacent regions have previously been shown in *Drosophila* and other organisms (Slatis 1955; John 1985; Hartmann *et al.* 2019a; Fernandes *et al.* 2024). Westphal and Reuter (2002) observed elevated centromere-proximal crossovers in a several suppressor-of-variegation mutants that impact chromatin structure. Three of the *Su(var)* mutants in their study mapped to genes encoding proteins necessary for heterochromatin formation and maintenance, including HP1 (*Su(var)2-5*) and H3K9 methyltransferase (*Su(var)3-9*), as well as their accessory proteins (*Su(var)3-7*). Peng and Karpen (2009) showed that a hetero-allelic *Su(var)3-9* mutant had elevated DSBs in meiotic cells that colocalized with alpha-heterochromatic sequences, suggesting that *Su(var)3-9* is crucial to keeping DSBs out of alpha heterochromatin during meiosis. Together, these data suggest that the inherent heterochromatic nature of large portions of the pericentromere contributes to crossover suppression within it.

In this study, we measured centromere-proximal crossover frequencies, the strength of the CE, and crossover distribution patterns within different regions of the pericentromere: proximal euchromatin, beta heterochromatin, and alpha heterochromatin (Fig. 1). We investigated 3 classes of mutants: those that contribute to SC structure, the process of meiotic recombination, and the establishment of heterochromatin. If different modes of crossover control are required to suppress crossovers in different centromere-proximal regions, we hypothesized that we would observe differences in where the CE is disrupted in each mutant class. The SC mutant we looked at was a *c(3)G* in-frame deletion that leads to failure to maintain full-length SC by mid-pachytene (Billmyre *et al.* 2019). We observed significant CE defects on chromosome 2 in this mutant, along with a considerable redistribution of crossovers away from proximal euchromatin, toward beta but not alpha heterochromatin. This suggests that full-length SC at mid-pachytene is required to suppress crossovers in beta heterochromatin. We also looked at mutants lacking MEI-218 and REC, which are crucial for crossover formation and patterning but have no known roles outside of meiosis (Carpenter and Baker 1982; Hartmann *et al.* 2019a). Upon establishing that both mutants have a significantly weakened CE, we found a significant increase in heterochromatic crossovers in both beta and alpha heterochromatin at the expense of crossovers in proximal euchromatin. Surprisingly, the heterochromatic mutant in our study—$Su(var)3\text{-}9^{null}$—turned out to be dispensable not only for centromere-proximal crossover suppression, but also for preventing crossovers specifically in pericentromeric heterochromatin, as no significant redistribution of crossovers was observed between proximal euchromatin and pericentromeric heterochromatin. As *Su(var)3-9* is a gene crucial for heterochromatinization at the pericentromere (Schotta *et al.* 2002) and is also implicated in preventing meiotic crossovers in heterochromatin (Westphal and Reuter 2002), this result implies that chromatin-based steric hindrance and inaccessibility do not play as big of a role in keeping crossovers out of heterochromatic regions as various classes of meiotic factors necessary for crossover designation and patterning do.

Our results suggest that while the cell seems to require multiple facets of control to exclude crossovers in centromere-proximal regions during meiosis, the CE is a primarily meiotic phenomenon in *Drosophila*, with the meiotic program—both the structure providing the conduit for proteins that carry out recombination and the recombination proteins themselves—seemingly superseding heterochromatin in preventing heterochromatic crossovers.

Materials and methods

Fly stocks

Flies were maintained at 25 °C on a corn meal-agar medium. The *Oregon-R* stock used as our wild-type control was generously provided by Dr. Scott Hawley. The *mei-218* mutant alleles used in this study ($mei\text{-}218^{1}$ and $mei\text{-}218^{6}$) are described in Baker and Carpenter (1972) and McKim *et al.* (1996). The *rec* mutant alleles used in this study (rec^{1} and rec^{2}) are described in Grell (1978), Matsubayashi and Yamamoto (2003), and Blanton *et al.* (2005). The *y; Su(var)3-*9^{06}*/TM3 Sr* and *y; Su(var)3-*9^{17}*/TM3 Sr* stocks were generously provided by Dr. Gary Karpen. The *y w/y + Y; c(3)*$G^{ccΔ2}$*/TM3, Sb; sv*$^{spa\text{-}pol}$ stock was generously provided by Dr. Katherine Billmyre. The presence of mutant alleles was verified where possible using allele-specific PCRs optimized for this purpose. Primer sequences are shown in Supplementary Table 2 in Supplementary File 2.

Fly crosses

Flies that were *Oregon-R* and *net* $dpp^{d\text{-}ho}$ *dp b pr cn* were isogenized, then incorporated into various mutant backgrounds. The following stocks were built for this study: *y* $mei\text{-}218^{1}$*/FM7 ; net-cn* iso/CyO, $mei\text{-}218^{6}$ *f/FM7 ; OR* + iso/CyO, *net-cn* iso/CyO; rec^{1} *Sb/TM6B Hu Tb, OR* + iso/CyO; *kar* ry^{606} rec^{2}*/MKRS Sb, OR* + iso/CyO; *Su(var)3-*9^{06}*/MKRS, Sb, net-cn* iso/CyO; *Su(var)3-*9^{17}*/MKRS Sb, y w; OR* + iso/CyO; *c(3)*$G^{ccΔ2}$*/MKRS, y w; net-cn* iso/CyO; *c(3)*$G^{ccΔ2}$*/TM6B*.

Recombination mapping

Meiotic crossovers were mapped on chromosome 2 by crossing females that were heterozygous for the markers *net* $dpp^{d\text{-}ho}$ *dp b pr* and *cn* in the mutant background of choice to males homozygous for the same markers. Mitotic crossovers were mapped by crossing males that were heterozygous for these markers on chromosome 2 and were *Su(var)3-*9^{06}*/+* or *Su(var)3-*9^{06}*/Su(var)3-*9^{17} chromosome 3 to females homozygous for the chromosome 2 markers. Males and females were both between 1 and 5 d old when mated, and each vial was flipped after 7 d. Progeny was scored for all phenotypic markers and any that had a pericentromeric crossover (between *pr* and *cn*) were collected to fine-map where within the pericentromere the crossover occurred, through allele-specific PCR. Complete datasets for all recombination mapping are given in Supplementary Table 1 in Supplementary File 2. Wild-type crossover distributions were taken from a previous recombination mapping dataset (Pazhayam *et al.* 2023). Total chromosome 2 crossover numbers for wild type were estimated using the same dataset, based on total proximal crossovers collected in this study ($n = 132$), and is indicated as "adjusted total crossovers" in Supplementary Table 1 in Supplementary File 2. For *c(3)*$G^{ccΔ2}$, fine-mapping of pericentromeric crossovers was done in 171 of the 478 flies with pericentromeric crossovers, requiring an adjusted total crossover number for percentages of total crossovers calculated in Table 1. This adjusted total crossover number is also indicated in Supplementary Table 1 in Supplementary File 2.

Table 1. Percentage of crossovers in the region of chromosome 2 being studied that occurred within each sub-section of the pericentromere sequence.

			Percentage of chromosome 2 crossovers		
Genotype	**Flies**	**Crossovers**	**Proximal euchromatin**	**Beta heterochromatin**	**Alpha heterochromatin**
Wild type	4331	2081	2.69	0.24	0
$c(3)G^{ccA2}$	5918	3788	4.05**	0.86***	0
$mei\text{-}218^{null}$	12,339	284	10.21***	3.87***	0.35
rec^{null}	16,776	848	10.97***	5.31***	0.94***
$Su(var)3\text{-}9^{06}$/+	10,154	4871	2.24	0.16	0
$Su(var)3\text{-}9^{null}$	8123	4289	2.98	0.37	0.02

n.s. $P > 0.01$, *$P < 0.01$, **$P < 0.002$, ***$P < 0.0002$ based 2-tailed Fisher's exact test between mutant and wild-type numbers of crossovers observed vs expected within the centromeric interval, corrected for multiple comparisons.

Recombination calculations

Genetic length was calculated in centiMorgans (cM) as follows: (r/n) * 100, where *r* represents the number of recombinant flies in an interval (including single, double, and triple crossovers) and *n* represents total flies that were scored for that genotype. Release 6.53 of the reference genome of *Drosophila* was used to calculate physical length between chromosome 2 markers used for phenotypic recombination mapping, based on locations in FlyBase (Öztürk-Çolak *et al.* 2024). Since alpha heterochromatin sequence is not yet assembled, we estimated the length from the estimated heterochromatic sequence, 5.4 Mb for 2*L* and 11.0 Mb for 2*R* (Adams *et al.* 2000), minus the length of beta heterochromatin sequence in the Release 6.53 assembly (1.39 Mb for 2*L*, 7.6 Mb for 2*R*). CE values were calculated as 1-(observed crossovers/expected crossovers). Expected crossovers = total crossovers in a genotype * (physical length of proximal interval/total physical length). Power analysis (for a one-sided hypothesis test with an alpha cut-off of 0.05) was done on total flies with a pericentromeric crossover for Fisher's exact tests between wild type and the mutants exhibiting a significant redistribution of crossover proportions within the pericentromere: $mei\text{-}218^{null}$, rec^{null}, and $c(3)G^{ccA2}$. The 2 outcomes we measured were proportion of pericentromeric crossovers in proximal euchromatin in mutant vs wild type and proportion of pericentromeric crossovers in beta + alpha heterochromatin in mutant vs wild type. For $mei\text{-}218^{null}$ ($n = 41$) vs wild type ($n = 132$), power was 0.91 for proximal euchromatic crossover proportions and 0.86 for heterochromatic crossover proportions. For rec^{null} ($n = 146$) vs wild type ($n = 132$), power was 1.00 for proximal euchromatic crossover proportions as well as heterochromatic crossover proportions. For $c(3)G^{ccA2}$ ($n = 171$) vs wild type ($n = 132$), power was 0.79 for proximal euchromatic crossover proportions as well as heterochromatic crossover proportions. Since power values are all ~0.8 or higher, we are able to confidently say that the effects we see in these mutants are not false positives. We also performed the same power analysis in the 2 mutants that did not show significant differences in pericentromeric crossover distributions ($Su(var)3\text{-}9^{null}$ and $Su(var)3\text{-}9^{06}$/+) and observed low power. This does not indicate a false negative at our current sample sizes, only that we cannot rule out the possibility of weak but significant effects in these mutants at greater sample sizes. However, we would likely not interpret any such effects as biologically significant.

SNPs defining pericentromeric regions

Illumina sequencing was done on isogenized stocks of *Oregon-R* and *net-cn* to identify SNP differences. DNA from ~50 whole flies was extracted using the QIAGEN DNeasy Blood and Tissue Kit and sequenced on the Illumina NovaSeq 6000. Reads were aligned to the reference genome using bowtie2 (v2.5.3) (Langmead and Salzberg 2012) and PCR and optical duplicates were marked using samtools markdup (v1.21) (Danecek *et al.* 2021). Variants were called using freebayes (v1.1.0) (Erik Garrisson 2012). Unique SNPs between the *net-cn* and *OR+* chromosome 2 were identified using bcftools isec (v1.20) (Danecek *et al.* 2021). SNPs were validated by analyzing reads using Integrative Genomics Viewer (Robinson *et al.* 2011) and via PCR.

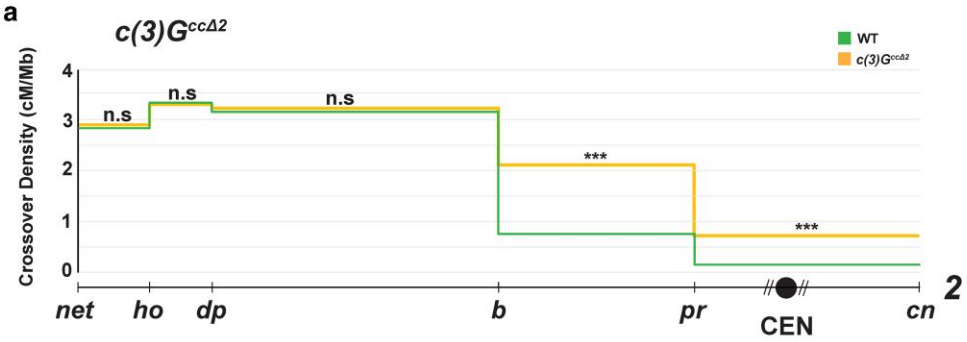
Four SNPs (called *beta2L*, *alpha2L*, *alpha2R*, and *beta2R*) were chosen to mark the boundaries between proximal euchromatin, beta heterochromatin, and alpha heterochromatin on each arm of chromosome 2. The *alpha2L* (position 23424573, C in *net-cn*, A in *OR+*) and *alpha2R* (position 639629, C in *net-cn*, A in *OR+*) SNPs chosen were the most proximal chromosome 2 SNPs in (Hartmann *et al.* 2019b). The *beta2L* (position 22036096, A in *net-cn*, T in *OR+*) and *beta2R* (position 5725487, C in *net-cn*, T in *OR+*) SNPs chosen were based on maximum proximity to the heterochromatin-euchromatin boundary as defined by various studies summarized in Supplementary Table 3 of Stutzman *et al.*(2024).

Proximal euchromatin is defined as the region between phenotypic marker *pr* and the *beta2L* SNP on chromosome 2*L* and the region between the *beta2R* SNP and the phenotypic marker *cn* on chromosome 2*R*. Beta heterochromatin is defined as the region between the *beta2L* SNP and *alpha2L* SNP on chromosome 2*L* and the *alpha2R* SNP and *beta2R* SNP on chromosome 2*R*. Alpha heterochromatin is defined as the region between the *alpha2L* SNP on chromosome 2*L* and the *alpha2R* SNP on chromosome 2*R*.

A second *beta2R* SNP (position 5726083, A in *net-cn*, T in *OR+*) was chosen for the progeny of $Su(var)3\text{-}9^{06}$/+ and $c(3)G^{ccA2}$ mutants with pericentromeric crossovers as the allele-specific PCR amplifying the *beta2R* SNP at position 5725487 was no longer robust toward the end of our study. For consistency, the progeny of wild type, $mei\text{-}218^{null}$, rec^{null}, and $Su(var)3\text{-}9^{06}/Su(var)3\text{-}9^{17}$ flies with pericentromeric crossovers where the position of the crossover was indicated by the presence or absence of the 5725487 *beta2R* band were re-confirmed with the allele-specific PCR amplifying the *beta2R* SNP at position 5726083. Additional SNPs *alpha2L_II* (position 23423662, A in *net-cn*, C in *OR+*) and *alpha2R_II* (position 637775, T in *net-cn*, C in *OR+*) were used to confirm each alpha-heterochromatic crossover that was observed. Primer sequences and PCR conditions are shown in Supplementary Table 3 in Supplementary File 2. Optimization PCRs for each SNP, as well as images of all gels on which SNPs were genotyped, are in Supplementary File 1 available on Figshare (doi https://doi.org/10.6084/m9.figshare.28079195).

Allele-specific PCR

Progeny from the crosses of experimental females of the desired mutant background and males homozygous for phenotypic



b

Genotype	Chromosome 2 CE Value
wild type	0.92
$c(3)G^{ccΔ2}$	0.65***

c

Genotype	Proportion of Pericentromeric Crossovers (%)		
	Proximal Euchromatin	Beta	Alpha
wild type	91.7	8.3	0
$c(3)G^{ccΔ2}$	82.5	17.5	0

Fig. 2. a) Crossovers in $c(3)G^{ccΔ2}$ ($n = 5{,}918$) and wild type ($n = 4{,}331$) flies along chromosome 2 with the Y-axis indicating crossover density in cM/Mb and the X-axis indicating physical distances between recessive marker alleles that were used for recombination mapping. The chromosome 2 centromere is indicated by a black circle, unassembled pericentromeric repetitive DNA by diagonal lines next to it. A 2-tailed Fisher's exact test was used to calculate statistical significance between mutant and wild-type numbers of total crossovers vs parentals in each interval. Complete dataset is in Supplementary Table 1 in Supplementary File 2. n.s. $P > 0.01$, *$P < 0.01$, **$P < 0.002$, ***$P < 0.0002$ after correction for multiple comparisons. b) Table showing CE values on chromosome 2 in wild type and $c(3)G^{ccΔ2}$ flies. A 2-tailed Fisher's exact test was performed between mutant and wild-type numbers of crossovers observed vs expected within the centromeric interval. n.s. $P > 0.01$, *$P < 0.01$, **$P < 0.002$, ***$P < 0.0002$ after correction for multiple comparisons. c) Table showing the percentage of pericentromeric crossovers that occurred within each region of the pericentromere in wild type vs $c(3)G^{ccΔ2}$ mutant flies. File S1 contains gel images of allele-specific PCRs for each SNP defining the boundaries of pericentromeric regions.

markers *net-cn* that had a pericentromeric crossover (a crossover between the most proximal markers *purple* and *cinnabar* on either arm of chromosome 2) were collected and DNA was extracted. Since the recombined chromosome from experimental females is recovered over a *net-cn* chromosome from males, all progeny carry the *net-cn* versions of each SNP. Therefore, allele-specific PCRs that amplify the *OR+* versions had to be performed on progeny with a pericentromeric crossover to map whether the crossover occurred in proximal euchromatin, beta heterochromatin, or alpha heterochromatin. For each allele-specific PCR, the presence of a band indicates that the recombined chromosome from the experimental female has the *OR+* version of the SNP. The absence of a band indicates that the recombined chromosome from the experimental female has the *net-cn* version of the SNP. With this information, we pinpointed the switch from *OR+* SNPs to *net-cn* SNPs on the recombined chromosome, telling us where the pericentromeric crossover in the experimental female occurred. Gels from all allele-specific PCRs for each fly of every genotype (wild type, $mei\text{-}218^{null}$, rec^{null}, and $Su(var)3\text{-}9^{06}/Su(var)3\text{-}9^{17}$, $Su(var)3\text{-}9^{06}/+$ and $c(3)G^{ccΔ2}$) are shown in Supplementary File 1.

Results

SC protein C(3)G is necessary for centromere-proximal crossover suppression during meiosis

The SC is a protein structure that forms specifically between paired homologs during meiosis. In *Drosophila*, the SC is formed before meiotic DSBs are induced, and plays a crucial role in both DSB and crossover formation (Page and Hawley 2001; Mehrotra and Mckim 2006; Lake and Hawley 2012; Collins *et al.* 2014), as well as crossover patterning (Billmyre *et al.* 2019). To ask how important the *Drosophila* SC is in establishing the CE, we measured recombination in a mutant defective for SC maintenance. $c(3)G^{ccΔ2}$ is a deletion the removes residues 346–361 from the coiled-coil domain of the transverse filament (Billmyre *et al.* 2019). This mutation results in the loss of the SC structure by mid-pachytene. Interestingly, $c(3)G^{ccΔ2}$ flies display elevated centromere-proximal crossovers on chromosome 3, which has a strong CE, but not on chromosome X, which has a weak CE, suggesting that C(3)G and a full-length SC are necessary to maintain a robust CE.

We asked whether C(3)G is important for pericentromeric crossover suppression on chromosome 2 as well by measuring crossover frequencies within a ~40 Mb region that spans the centromere and includes euchromatin, beta heterochromatin, and alpha heterochromatin. Female flies heterozygous for markers on both arms of chromosome 2 were used to map recombination between the distal 2*L* locus *net* and the proximal 2*R* locus *cinnabar* (*cn*). The centromere on chromosome 2 lies in the interval between markers *purple* (*pr*) on 2*L* and *cn* on 2*R*, covering an approximate length of 20.5 Mb, including 11.2 Mb of assembled sequence and an estimated 4 Mb of alpha heterochromatin on 2*L* and 5.3 Mb on 2*R*.

Figure 2a shows crossover density along chromosome 2 (divided into 5 intervals by 6 recessive marker alleles) in wild-type flies and in $c(3)G^{ccΔ2}$ mutants. Total genetic length in this mutant is significantly increased in the mutant, from 48.05 cM in wild type to 64.01 cM ($P < 0.0001$). While crossover distributions closely resemble wild type in the 3 distal and medial intervals interval 2, crossover frequencies in the interval spanning the centromere (*pr* - *cn*) and the adjacent interval (*b* - *pr*) are significantly increased in the $c(3)G^{ccΔ2}$ mutant ($P < 0.0001$; Fig. 2a). This suggests that chromosome 2, like chromosome 3, experiences a weaker than wild-type CE in this mutant.

Since crossover frequencies measured in cM/Mb are based only on observed crossover numbers, we calculated a CE value that also takes into account crossover numbers expected if there

were no centromere-proximal suppression during meiotic recombination. This value (expanded upon in the Methods section) considers crossover density in the centromeric interval as equal to the average density of the entire chromosome 2 region being studied and is a more biologically relevant measure of the CE as it is agnostic to differences in total crossover numbers between 2 genotypes.

Wild-type flies have a CE value of 0.92 on chromosome 2 (Pazhayam *et al.* 2024), whereas the $c(3)G^{ccΔ2}$ mutant has a significantly lower CE value of 0.65 ($P < 0.0001$; Fig. 2b), consistent with a strong defect in the CE. This suggests that the maintenance of full-length SC throughout pachytene is essential for ensuring vigorous suppression of centromere-proximal meiotic crossovers in *Drosophila*.

The SC protein C(3)G is necessary for crossover suppression in beta but not alpha heterochromatin

On observing that the *Drosophila* SC component C(3)G is crucial for centromere-proximal crossover suppression on chromosome 2, we asked whether it plays a role in the distribution of crossovers across the various regions of the pericentromere. To determine this, we built flies of the desired mutant background that were heterozygous for isogenized *net-cn* and wild-type chromosomes. We identified SNPs between these chromosomes that we used to fine-map crossovers within the larger intervals defined by phenotypic markers. We collected every fly that had a crossover between *pr* and *cn* and used allele-specific PCR to map the crossover to proximal euchromatin or beta heterochromatin on either arm, or to alpha heterochromatin. Here, alpha heterochromatin is defined as the region between the most proximal SNPs on 2*L* and 2*R* of the current assembly (release 6.59 of the *D. melanogaster* reference genome). This includes the centromere and pericentromeric satellite DNA. Beta heterochromatin is defined as the region on either chromosome arm between the most proximal SNP and the position where the H3K9me3 heterochromatin mark ends (Stutzman *et al.* 2024). Proximal euchromatin is defined as the region between the heterochromatin/euchromatin boundary and the visible phenotypic marker we used on each arm. It should be noted that the current assembly of the *Drosophila* reference genome is incomplete, so the regions we define as alpha heterochromatin may contain some unassembled beta heterochromatin. Since no crossovers were recovered between the most proximal SNPs in wild-type flies in this study or a similar study (Hartmann *et al.* 2019b), the existence of any in crossovers in this region is noteworthy. Double crossovers cannot be recovered using our approach, although we expect these to be vanishingly rare.

Intriguingly, the $c(3)G^{ccΔ2}$ mutant displayed a significant redistribution of crossovers across 2 of the 3 proximal regions. The distribution in this mutant, measured as percentages of total crossovers across the chromosomal region being studied, was significantly increased from wild type in proximal euchromatin and beta heterochromatin (Table 1). While only ~2.7% of total crossovers on chromosome 2 form in proximal euchromatin in wild-type flies, $c(3)G^{ccΔ2}$ mutants had ~4.1% of total chromosome 2 crossovers now found in this region ($P = 0.0012$). Similarly, ~0.9% crossovers in $c(3)G^{ccΔ2}$ mutants are found in beta heterochromatin, a significant increase from the ~0.2% observed in wild-type flies ($P = 0.0002$; Table 1). Curiously, we observed no crossovers mapping to the region between our most proximal SNPs on 2*L* and 2*R*, meaning that no crossovers occurred in alpha heterochromatin, as in wild-type flies (Table 1). This suggests that while SC mutants are unable to maintain wild-type levels of crossover suppression in beta heterochromatin, they are as successful as wild-type flies in suppressing crossovers in alpha heterochromatin.

We calculated crossover frequencies in each region of the pericentromere as a percentage of total pericentromeric crossovers in this mutant (Fig. 2c), and observed a statistically significant redistribution from proximal euchromatin toward beta ($P = 0.0268$) but not alpha heterochromatin ($P = 1.000$), compared with wild type.

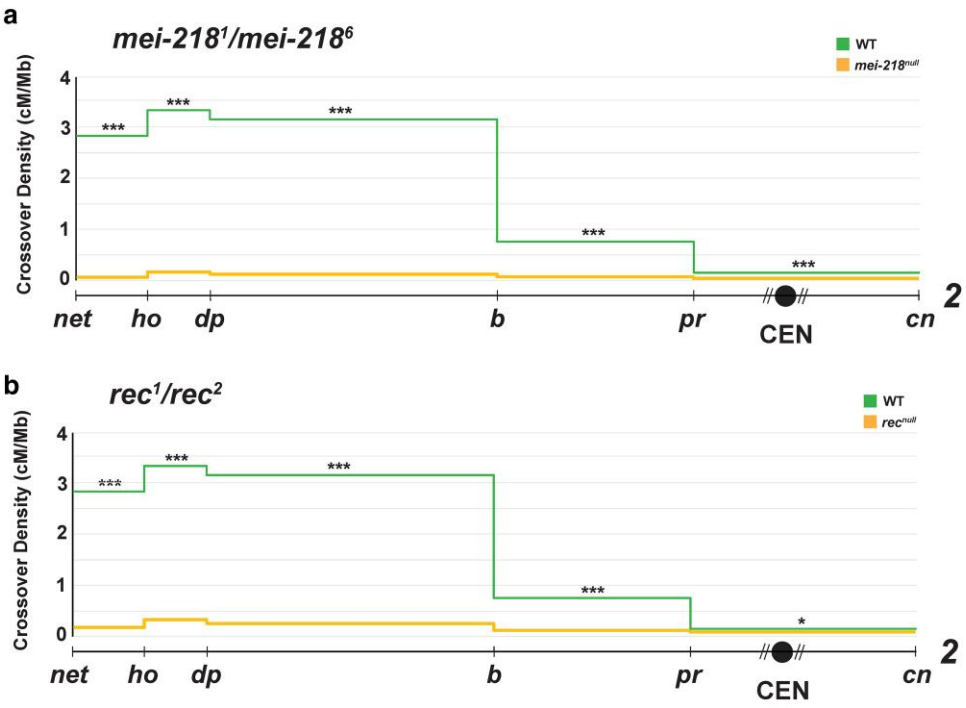
Collectively, these data indicate that full-length SC during mid-pachytene plays a role in maintaining wild-type levels of crossover suppression at the pericentromere (Fig. 2a and b) as well as wild-type proportions of crossovers within proximal euchromatin and beta heterochromatin but is dispensable for crossover suppression within alpha heterochromatin (Fig. 2c, Table 1).

Meiotic recombination genes are necessary for centromere-proximal crossover suppression

Crossovers during meiosis are controlled by a meiotic program that designates and likely also patterns their formation along the length of the chromosome. To measure the influence of the meiotic program on centromere-proximal crossover suppression and the strength of the CE, we first looked at a null mutant of *mei-218*, which encodes a component of the meiotic-mini-chromosome maintenance (mei-MCM) complex required to make crossovers (Kohl *et al.* 2012). Mei-218 is crucial for the formation and patterning of meiotic crossovers (Baker and Carpenter 1972; Brand *et al.* 2018; Hartmann *et al.* 2019a). We addressed the role of *mei-218* in exerting the CE by measuring recombination along chromosome 2, between *net* and *cn*. Crossover density in *mei-218* null mutants is shown in Fig. 3a. Consistent with its crucial role in crossover formation during meiosis, the *mei-218* mutant had a significantly reduced genetic length (2.30 cM, $P < 0.0001$) along the chromosome 2 region being studied than wild-type flies did (48.05 cM). Notably, the distribution of crossovers along the chromosome in *mei-218* mutants appears to be almost flat, substantially different from the usual bell curve observed in wild-type flies. The genetic length of the interval containing the centromere was very similar to the genetic length of intervals along the rest of the chromosome in this mutant, indicating an impaired CE (Fig. 3a, complete dataset in Supplementary Table 1 in Supplementary File 2).

The *mei-218* mutant had a CE value of 0.60 on chromosome 2 (Fig. 3c), a significant decrease from the wild-type chromosome 2 CE value of 0.92 ($P < 0.0001$), further suggesting a very weak CE in this mutant, consistent with what was observed by Hartman *et al.* (2019a). Combined with the flat distribution of crossovers observed in this mutant, *mei-218* appears to be essential in establishing a robust suppression of crossovers near the centromere during meiosis.

To ask whether this importance in centromere-proximal crossover suppression extended to other pro-crossover meiotic genes, we also studied mutants defective for *rec*, which encodes another mei-MCM component (Kohl *et al.* 2012). Figure 3b shows crossover density along chromosome 2 in *rec* null mutants, which also have a significant decrease in genetic length (5.05 cM; $P < 0.0001$) from the wild-type level. Crossovers in this mutant followed the pattern of the *mei-218* mutant, with a much flatter distribution observed along the chromosome than in wild-type flies. The genetic length of the interval spanning the centromere was once again higher than or much closer to the genetic lengths of intervals in the middle of the chromosome arm, suggesting that *rec* mutants also have a diminished CE. This is further corroborated by the CE value of *rec* mutant flies (0.52), significantly reduced from wild-type chromosome 2 CE value of 0.92 ($P < 0.0001$) (Fig. 3c), indicating that Rec



c

Genotype	Chromosome 2 CE Value
wild type	0.92
mei-218 null	0.60***
rec null	0.52***

d

Genotype	Proportion of Pericentromeric Crossovers (%)		
	Proximal Euchromatin	Beta	Alpha
wild type	91.7	8.3	0
mei-218 null	70.7	26.8	2.4
rec null	63.7	30.8	5.5

Fig. 3. a) Crossovers in *mei-218*^{null} ($n = 12{,}339$) and wild-type ($n = 4{,}331$) flies along chromosome 2 with the Y-axis indicating crossover density in cM/Mb and the X-axis indicating physical distances between recessive marker alleles that were used for recombination mapping. The chromosome 2 centromere is indicated by a black circle, unassembled pericentromeric repetitive DNA by diagonal lines. b) Crossovers in *rec*^{null} ($n = 16{,}776$) and wild type ($n = 4{,}331$). c) CE values on chromosome 2 in wild-type, *mei-218*^{null}, and *rec*^{null} flies. d) Table showing percentage of pericentromeric crossovers that occurred within each region of the pericentromere in wild-type, *mei-218*^{null}, and *rec*^{null} flies. For all panels, a 2-tailed Fisher's exact test was used to calculate statistical significance between mutant and wild-type numbers of total recombinant vs nonrecombinants in each interval (see Supplementary Table 1 in Supplementary File 2 for complete datasets). n.s. $P > 0.01$, *$P < 0.01$, **$P < 0.002$, ***$P < 0.0002$, after correction for multiple comparisons. File S1 contains gel images of allele-specific PCRs for each SNP defining the boundaries of pericentromeric regions.

is also crucial for maintaining a strong CE. Overall, these results demonstrate that genes encoding 2 components of the mei-MCM complex—Mei-218 and Rec—are independently necessary to ensure that crossovers form at the right frequencies, and to guarantee centromere-proximal crossover suppression in *Drosophila*.

Meiotic recombination genes are necessary for crossover suppression in alpha and beta heterochromatin

On observing that the meiotic mutants *rec* and *mei-218* both have an ablated CE, we asked whether these genes are also necessary to maintain wild-type patterns of crossover distribution within the pericentromere. Hartmann *et al.* (2019b) previously fine-mapped centromere-proximal crossovers in *Blm* mutants, which also lack a functional CE, and observed a flat crossover distribution that extended into proximal euchromatin and beta heterochromatin, but never into alpha heterochromatin. They concluded that Blm is necessary to maintain the distance-dependent CE observed in beta heterochromatin and proximal euchromatin, but that the complete suppression of crossovers observed in alpha heterochromatin is likely due to the region not being under genetic/meiotic control, hypothesizing instead that highly repetitive regions do not experiencing meiotic DSBs.

This pattern of crossover redistribution in *Blm* mutants is similar to what we observed in the SC mutant *c(3)G*^{ccΔ2}, consistent with an important contribution of the SC in regulating meiotic recombination. Since the CE is weakened in both *rec* and *mei-218* mutants, much like in *Blm* and *c(3)G*^{ccΔ2} mutants, we sought to ask whether fine mapping crossovers within the pericentromere in *mei-218* and *rec* mutants would reveal the same patterns of crossover redistribution observed in *Blm*^{null} and *c(3)G*^{ccΔ2} flies. Surprisingly, pericentromeric crossover distribution patterns in the *mei-218* and *rec* mutants were different from both *Blm* and *c(3)G*^{ccΔ2} mutants. In *mei-218* mutants, 10.2% of total chromosome 2 crossovers were within proximal euchromatin, a significant increase from both the wild-type value of 2.7% in this region, as well as the *c(3)G*^{ccΔ2} value of 4.05% ($P < 0.0001$ for both comparisons). Similarly, 3.9% of total crossovers in *mei-218* mutants form in beta heterochromatin, also a significant increase compared with wild type ($P < 0.0001$) and *c(3)G*^{ccΔ2} ($P = 0.0002$) flies (Table 1).

Interestingly, we observed an increase in crossover frequencies in the region described as alpha heterochromatin, with 0.4% of total chromosome 2 crossovers in *mei-218* mutants forming between

our most proximal SNPs, compared with none in both wild-type and SC mutant flies (Table 1). The increase isn't statistically significant ($P = 0.35$), but statistical power is limited by the severe reduction in total crossovers in *mei-218* leading to few pericentromeric crossovers (41 from >12,000 flies scored). Because we never saw a crossover between the most proximal SNPs in wild type ($n = 132$), the increase observed in the *mei–218* mutant may be biologically relevant.

We then looked at pericentromeric crossover distributions in the *rec* mutant and observed similar patterns to those of the *mei-218* mutant. When compared with wild type, crossover frequencies, measured as a percentage of total crossovers across chromosome 2, were increased in all 3 regions of *rec* mutants (Table 1). Crossover frequencies increased to ~11% in proximal euchromatin, ~5.3% in beta heterochromatin, and ~0.9% in alpha heterochromatin, all significant ($P < 0.0001$) changes from crossover frequencies in the respective pericentromeric regions of wild-type and SC mutant flies.

We also calculated crossover frequencies as a percentage of total pericentromeric crossovers (Fig. 3d) and observed a statistically significant redistribution from proximal euchromatin toward beta heterochromatin in both *mei-218* ($P = 0.0049$) and *rec* ($P < 0.0001$) mutants, compared with wild-type flies. Compared with $c(3)G^{cc42}$ flies, *mei-218* mutants did not exhibit a significant redistribution of crossovers from proximal euchromatin to beta heterochromatin ($P = 0.1824$), but *rec* mutants did ($P = 0.0032$). *rec* mutant flies also displayed a highly significant redistribution of pericentromeric crossovers from proximal euchromatic regions toward alpha heterochromatin, compared with both wild-type ($P = 0.0016$) and SC mutant ($P = 0.0008$) flies.

Collectively, these results suggest that when the mei-MCM complex is lost, there is a significant repositioning of crossovers within the pericentromere, compared with both wild-type and the SC mutant in our study. More specifically, we observe a clear redistribution of pericentromeric crossovers away from proximal euchromatin and into both alpha and beta heterochromatin. Centromere-proximal crossovers in both mutants can reach further into pericentromeric heterochromatin than in wild-type, *Blm* mutant, or SC mutant flies, indicating not only a weakening of the strength of the CE but also its reach along the chromosome. This is particularly striking, as heterochromatic crossover suppression has been widely thought to happen through nonmeiotic mechanisms (Carpenter and Baker 1982; Szauter 1984; Westphal and Reuter 2002; Mehrotra and Mckim 2006), possibly through heterochromatinization and steric hindrances to DSB and recombination machinery. We had expected to see increases in crossovers within pericentromeric heterochromatin only in mutants of important heterochromatin genes. Instead, crossovers within heterochromatin seem to be unambiguously under meiotic control.

Su(var)3-9 is dispensable for centromere-proximal crossover suppression during meiosis

On observing that the meiotic machinery—in the form of both SC and recombination proteins—is necessary to prevent heterochromatic crossovers, we asked what pericentromeric crossover distributions look like in a heterochromatin mutant. As the majority of the chromosomal region described as the pericentromere is heterochromatic, we wanted to investigate whether mutations in genes necessary for heterochromatin formation and maintenance disrupt the CE and/or the suppression of heterochromatic crossovers to even greater extents than observed in our SC and meiotic recombination mutants.

To this end, we wished to look at a some of the suppressor of variegation mutants that were reported to have elevated centromere-proximal crossovers (Westphal and Reuter 2002). Of the genes in that study, *Su(var)3-7* and *Su(var)3-9* were of the most interest to us, as they encode critical heterochromatin-associated proteins. *Su(var)3-9* codes for the H3K9 methyltransferase responsible for methylating pericentromeric heterochromatin, and SU(VAR)3–7 functions as an HP1 companion (Cléard *et al.* 1997; Delattre *et al.* 2000) and potential anchor for the HP1 and SU(VAR)3-9 complex (Westphal and Reuter 2002).

We hypothesized that the elevation of pericentromeric crossovers observed on chromosome 3 in the *Su(var)3-7* heterozygote and the *Su(var)3-7 Su(var)3-9* double heterozygote in (Westphal and Reuter 2002) would hold true on chromosome 2, and that the excess centromere-proximal crossovers in these mutants would map to the heterochromatic regions of the pericentromere. We assayed flies with a heteroallelic *Su(var)3-9* genotype previously observed to have elevated DSBs in female meiotic cells (Peng and Karpen 2009). We hypothesized that this elevation would lead to an increase in centromere-proximal crossovers and a subsequent weakening of the CE.

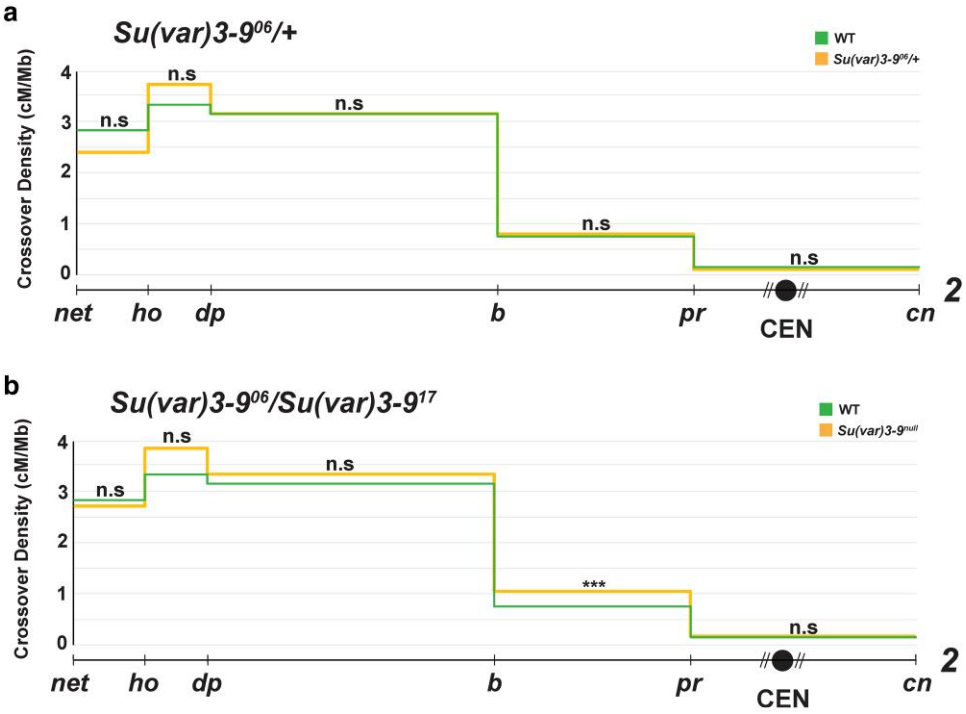
When crossover distribution was measured along chromosome 2 in $Su(var)3\text{–}9^{06}/Su(var)3\text{–}9^{17}$ females, we found an increase in genetic length in the region being studied, from 48.05 cM in wild-type females to 52.8 cM in the mutant ($P = 0.0041$); however, this elevation in genetic length comes from an increase in distal, euchromatic crossovers that lie outside of the purview of SU(VAR)3-9's H3K9 methylation functions. Furthermore, crossover frequencies within the interval containing the centromere were not different from wild-type levels, and no change in crossover density was observed (Fig. 4b). The chromosome 2 CE value in this mutant (0.91) was also unchanged from the wild-type chromosome 2 CE value (0.92) (Fig. 4c), further indicating that the CE remains intact. This is despite the reported elevation in DSBs in meiotic cells in this mutant (Peng and Karpen 2009). This suggests that crossover homeostasis is intact in this mutant, consistent with meiotic cells employing multiple levels of control to ensure crossover suppression around the centromere.

We also measured crossover distribution along chromosome 2 in a $Su(var)3\text{-}9^{06}/+$ heterozygote (Fig. 4a) and observed no changes from wild type in total genetic length (47.97 cM) or in crossover density in the centromeric interval. The $Su(var)3\text{-}9^{06}$ heterozygote had a CE value of 0.93 (Fig. 4c), not significantly different from the wild-type CE value of 0.92 ($P = 0.2050$), indicating that the CE remains robust in this mutant.

Collectively, these results demonstrate that the H3K9 methyltransferase necessary for the heterochromatinization of pericentromeres is dispensable both for the formation of crossovers and for the suppression of crossovers in pericentromeric regions. Crossover homeostasis and CE machinery are reliably able to function in these mutants to guarantee that crossovers form at the correct frequencies and in the right chromosomal regions.

Su(var)3-9 is dispensable for suppressing crossovers in heterochromatin

Although no changes were observed in the strength of the CE in *Su(var)3-9* mutants, it is still possible that crossover distribution within the pericentromeric interval is affected. Peng and Karpen (2009) reported in 2009 that many of the excess DSBs they observed in meiotic cells of $Su(var)3\text{-}9^{06}/Su(var)3\text{-}9^{17}$ mutants co-localized



c

Genotype	Chromosome 2 CE Value
wild type	0.92
3-9^06 / +	0.93
3-9^06 / 3-9^17	0.91

d

Genotype	Proportion of Pericentromeric Crossovers (%)		
	Proximal Euchromatin	Beta	Alpha
wild type	91.7	8.3	0
3-9^06 / +	93.2	6.8	0
3-9^06 / 3-9^17	88.3	11.0	0.7

Fig. 4. a) Crossovers in *Su(var)*3-9^{06}/+ ($n = 10{,}154$) and wild-type ($n = 4{,}331$) flies along chromosome 2 with the Y-axis indicating crossover density in cM/Mb and the X-axis indicating physical distances between recessive marker alleles that were used for recombination mapping. The chromosome 2 centromere is indicated by a black circle, unassembled pericentromeric DNA by diagonal lines. b) Crossovers in *Su(var)*3-9^{06}/*Su(var)*3-9^{17} ($n = 8{,}123$) and wild-type ($n = 4{,}331$) flies. c) CE values on chromosome 2 in wild-type, *Su(var)*3-9^{06}/+, and *Su(var)*3-9^{06}/*Su(var)*3-9^{17} flies. d) Percentages of pericentromeric crossovers that occurred within each region of the pericentromere in wild-type, *Su(var)*3-9^{06}/+, and *Su(var)*3-9^{06}/*Su(var)*3-9^{17} flies. For all panels, a 2-tailed Fisher's exact test was used to calculate statistical significance between mutant and wild-type numbers of total crossovers vs nonrecombinants in each interval. n.s. $P > 0.01$, $^{*}P < 0.01$, $^{**}P < 0.002$, $^{***}P < 0.0002$ after correction for multiple comparisons. Supplementary Table 1 in Supplementary File 2 contains complete datasets. Supplementary File 1 contains gel images of allele-specific PCRs for each SNP defining the boundaries of pericentromeric regions.

with signals from fluorescent in situ hybridization of probes to satellite DNA sequences, something never seen in wild-type flies. This suggests that there may be a redistribution of crossovers within the pericentromeric interval toward alpha-heterochromatic regions. However, when we measured crossover frequencies in the *Su(var)*3-9^{06}/*Su(var)*3-9^{17} mutant in each of the pericentromeric regions (as a percent of total crossovers across the chromosomal region being studied) we found that they closely resembled wild-type levels (Table 1), with ~3% of total crossovers on chromosome 2 forming in proximal euchromatin and ~0.4% forming in beta heterochromatin. These are not significant changes from wild-type percentages ($P = 0.4406$ and 0.3363, respectively).

We also calculated crossover frequencies within each pericentromeric region as a percentage of total crossovers within the pericentromere, and once again observed no significant changes from wild-type frequencies, with 88% of pericentromeric crossovers mapping to proximal euchromatin ($P = 0.8614$ compared with wild type) and 11% to beta heterochromatin ($P = 0.5486$) (Fig. 4d). However, we did observe one crossover between the most proximal SNPs, which we never saw in our dataset from wild-type females.

We also looked at pericentromeric crossover distributions in the *Su(var)*3-9 heterozygote tested by Westphal and Reuter (2002), but saw no significant changes in total or pericentromeric crossover frequencies in proximal euchromatin, beta heterochromatin, or alpha heterochromatin. As in wild-type flies, 2.2% of total crossovers in this mutant were in proximal euchromatin, 0.2% were in beta heterochromatin, and 0% were in alpha heterochromatin (Table 1). Percentages of total pericentromeric crossovers also closely resembled wild-type percentages, with 93.2% occurring in proximal euchromatin and 6.8% occurring in beta heterochromatin (Fig. 4d).

Overall, the lack of any significant redistribution of crossovers within the pericentromere tells us that meiosis is successfully able to suppress pericentromeric crossovers in *Su(var)*3–9^{06}/*Su(var)*3-9^{17} mutants. Peng and Karpen (2007) showed that this mutant has reduced H3K9 methylation at repetitive regions of the genome, suggesting that H3K9 methylation—a hallmark of heterochromatinization—within the pericentromere is surprisingly dispensable for crossover suppression in beta heterochromatin and for keeping pericentric crossovers within proximal euchromatin. It also appears to be largely or completely dispensable for crossover suppression in alpha heterochromatin. Despite allowing for more heterochromatic DSBs during meiosis, the *Su(var)*3-9 mutant can maintain wild-type distributions of

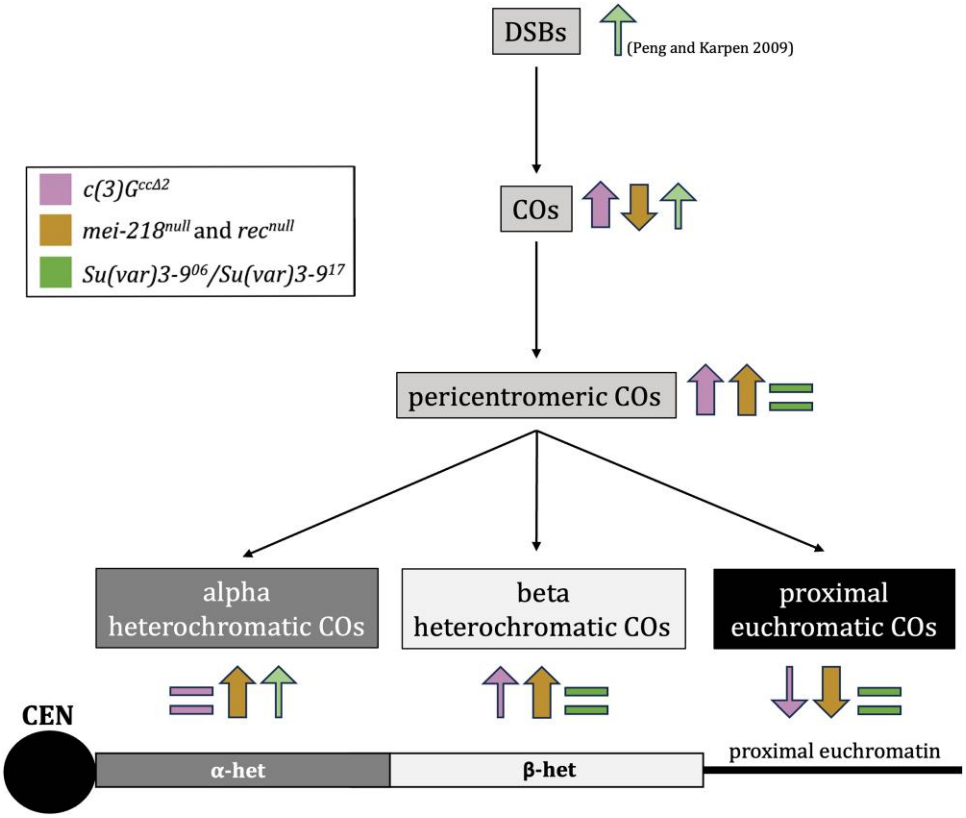


Fig. 5. Summary of the effects of each mutant in this study on the formation of DSBs, crossovers, pericentromeric crossovers, alpha-heterochromatic crossovers, beta-heterochromatic crossovers, and proximal euchromatic crossovers. The arrows indicate whether there is an increase or decrease in the indicated event, with colors denoting the mutant in question. Purple is *c(3)G*^{ccΔ2}, dark yellow is *mei-218*^{null} and *rec*^{null} combined, green is *Su(var)3-9*^{06}/*Su(var)3-9*^{17}. Thickness of the arrows and intensity of color indicate the strength of the increase/decrease. A schematic of a telocentric chromosome is shown below, with the centromere, alpha heterochromatin, beta- heterochromatin, and proximal euchromatin indicated.

crossovers within the *Drosophila* pericentromere, completely unlike the SC and meiotic recombination mutants in our study.

Discussion

Previous studies have shown that the CE manifests differently in different regions of the pericentromere, with alpha heterochromatin displaying no crossovers and beta heterochromatin and proximal euchromatin displaying crossover suppression that diminishes with increasing distance from the centromere (Hartmann *et al.* 2019b; Fernandes *et al.* 2024). This suggests that the CE may be established via distinct mechanisms in different pericentromeric regions, motivating us to look at patterns of centromere-proximal crossover formation in 3 classes of mutants. These mutants affect either SC maintenance (Billmyre *et al.* 2019), meiotic recombination (Baker and Carpenter 1972; Hartmann *et al.* 2019a), or heterochromatin formation (Schotta *et al.* 2002), and were used to ask whether each of these processes exerts control over crossover suppression in independent regions of the pericentromere

Our data show that crossover regulation at the pericentromere is indeed multifaceted, with each class of mutants exhibiting distinct patterns of crossover formation in the various pericentromeric regions, summarized in Fig. 5. We discuss the mechanistic implications of these results below.

SC and the CE

The SC is a meiotic structure essential for recombination in *Drosophila*, possibly by facilitating the movement of meiotic recombination factors along chromosomes. It provides a framework of sorts for the process of crossing-over and has been shown to contribute toward crossover patterning in various ways (Sym and Roeder 1994; Wang *et al.* 2015; Billmyre *et al.* 2019; Zhang *et al.* 2021). We sought to ask how disrupting the SC affects pericentromeric crossover suppression and distribution.

The SC mutant in our study is an in-frame deletion of *c(3)G*, which encodes the transverse filament of the *Drosophila* SC and is essential for SC assembly as well as meiotic recombination (Page and Hawley 2001). The allele we used—*c(3)G*^{ccΔ2}—has defects in SC maintenance and fails to retain its full-length structure by mid-pachytene (Billmyre *et al.* 2019). This mutation was also shown to cause increased centromere-proximal crossovers on chromosome 3, making it an ideal candidate to test how the SC contributes to the CE as well as to suppressing crossovers in different regions of the pericentromere.

Our data show the *c(3)G* mutant having a significantly weaker CE (Fig. 2a and b) as well as a pericentromeric crossover redistribution phenotype that is intermediate between our meiotic recombination mutants and wild-type flies. While a significant increase in percentage of total crossovers is observed in both proximal euchromatin and beta heterochromatin in *c(3)G*^{ccΔ2} flies, no change is observed in alpha-heterochromatic crossover frequencies when compared with wild type (Table 1). Additionally, the increases observed in proximal euchromatin and beta heterochromatin in the SC mutant do not reach the levels observed in either meiotic mutant (Table 1, Figs. 2c and 3c), indicating that while full-length SC during mid-pachytene is necessary for

centromere-proximal crossover suppression and to maintain wild-type proportions of crossovers within proximal euchromatin and beta heterochromatin, it doesn't appear to be as crucial as the meiotic-MCM genes.

This is surprising as it tells us that despite $c(3)G^{ccΔ2}$ mutants having an ablated CE, meiotic cells in this mutant are still able to regulate crossover formation within the pericentromere and prevent the spread of excess centromere-proximal crossovers into alpha heterochromatin, and even into beta heterochromatin at the levels allowed in *mei-218* and *rec* mutants. Like Blm, C(3)G appears to be necessary to maintain the distance-dependent CE observed in beta heterochromatin and proximal euchromatin, but dispensable for the complete suppression observed in alpha heterochromatin. These data suggest that it is possible to disrupt the CE in different ways—using different classes of mutants—that may allow an increase in crossovers within one region of the pericentromere but not another, or even different levels of crossover increases within the same region.

Our observations also fit well with the SC serving as a conduit for the recombination proteins that designate and pattern crossovers during prophase I (Zhang *et al.* 2021; Fozard *et al.* 2023; von Diezmann *et al.* 2024). Without any SC, as in the case of *c(3)G* null mutants, flies are completely unable to make meiotic crossovers (Page and Hawley 2001). This could be because meiotic proteins now lack a phase through which to travel along the length of paired homologs. In the $c(3)G^{ccΔ2}$ mutant, however, crossovers still form—at rates even higher than in wild type—but the CE is drastically weakened, which suggests that meiotic proteins can diffuse enough to designate crossovers along the chromosome, but somehow lose the ability to suppress them at the pericentromere. One explanation for this could be that centromere-proximal crossover suppression might be enforced after initial crossover designation. The $c(3)G^{ccΔ2}$ mutant has full-length SC in early and early/mid-pachytene, but this is lost by mid-pachytene. It is possible that initial crossover designation occurs in early-pachytene, but the CE is established in mid-pachytene, and therefore severely disrupted in this mutant. Crossover distribution patterns being altered in $c(3)G^{ccΔ2}$ flies could also be related to timing, as it is possible that crossover suppression in alpha heterochromatin happens early, when the SC in these mutants is still fully intact, with beta-heterochromatic and proximal euchromatic crossovers being suppressed at mid-pachytene or later, when full-length SC is lost in the mutant. Measuring the strength of the CE as well as pericentromeric crossover patterns in the other deletion mutants described in (Billmyre *et al.* 2019) that lose full-length SC at different times during pachytene could shed light on which ones are important for crossover suppression in the different pericentromeric regions.

An interesting point to note about the $c(3)G^{ccΔ2}$ mutant is that while it has a weaker than wild-type CE on chromosomes 2 and 3, the weak CE on the X chromosome appears not to be affected (Billmyre *et al.* 2019). Curiously, another *c(3)G* deletion described by Billmyre *et al.* (2019)—$c(3)G^{ccΔ1}$—displays CE defects on all 3 chromosomes, suggesting that different aspects of SC function and maintenance are important for CE establishment on different chromosomes. This suggests that CE mechanisms may not be uniform across the genome. Investigating how pericentromeric crossover distributions are changed in *c(3)G* mutants that have an ablated CE on all 3 chromosomes may illuminate which aspects of SC function are important across the board, and which are important only for certain chromosomes.

Recombination machinery and the CE

The recombination genes in our study—*mei-218* and *rec*—encode 2 major components of the mei-MCM complex, a pro-crossover protein complex necessary for both crossover formation and patterning during meiosis (Kohl *et al.* 2012). As these proteins are crucial for meiotic recombination but have no SC defects (Carpenter 1979), they provide data that is easily separable from the $c(3)G^{ccΔ2}$ mutant, allowing us to draw conclusions about the importance of recombination machinery independently of SC-mediated influences to centromere-proximal crossover suppression.

Based on data from the SC mutant in our study, as well as *Blm* mutants (Hatkevich *et al.* 2017), we hypothesized that *mei-218* and *rec* mutants would exhibit a similarly defective CE, with increased pericentromeric crossovers in proximal euchromatin and beta heterochromatin but no changes from the complete crossover suppression in alpha heterochromatin. While we did observe significantly weaker CEs in both recombination mutants, we were surprised to see a substantial increase of total crossover percentages across all 3 regions of the pericentromere, with a significant redistribution of crossovers away from proximal euchromatin toward both beta and alpha heterochromatin (Table 1; Fig. 3d). It is noteworthy that both *mei-218* and *rec* mutants have crossovers between our most proximal SNPs, as none were ever observed in *Blm* or *c(3)G* mutants (Hartmann *et al.* 2019; Fig. 3d). This suggests that the mei-MCM complex suppresses crossovers within alpha heterochromatin and/or deeper into beta heterochromatin than Blm or SC. These data also indicate that these 2 parts of the meiotic recombination machinery may have distinct areas of control within the pericentromere. Pericentromeric crossover distributions in double mutants could shed light on whether Blm and the mei-MCM complex work in tandem to maintain the CE and are equally important to suppress crossovers in the region.

Aside from how crossover distribution in these mutants differs from the *Blm* and *c(3)G* mutant, it is also unexpected and noteworthy that Mei-218 and Rec are necessary to prevent crossovers in heterochromatin. Previous data has shown that while "recombination-defective meiotic mutants" such as *mei-218* can change euchromatic crossover distribution patterns on chromosome X and, unexpectedly, 4, they do not allow for the formation of heterochromatic crossovers on either chromosome (Sandler and Szauter 1978; Carpenter and Baker 1982). Szauter (1984) inferred that the mechanisms "that prevent crossovers in heterochromatin are distinct from those that specify the distribution of crossovers in the euchromatin" (Szauter 1984). Our chromosome 2 results appear to contradict these conclusions, showing not only that heterochromatic crossovers *can* be under the control of meiotic machinery in *Drosophila*, but also reinforcing our hypothesis that the CE is mediated differently on different chromosomes.

Heterochromatin and the CE

While both facets of the meiotic machinery tested in our study—SC and recombination genes—were observed to suppress heterochromatic crossovers, we wondered whether a stronger influence on pericentromeric crossover suppression is exerted by genes essential for heterochromatin formation, given that much of the pericentromere is heterochromatic. To test this, we used mutants of *Su(var)3-9*, which encodes the H3K9 methyltransferase that methylates and aids in the heterochromatinization of the pericentromere. Specifically, we tested a *Su(var)3-9* heterozygote

—*Su(var)3-9*^{06}/+—as well as a heteroallelic null mutant *Su(var)3–9*^{06}/*Su(var)3-9*^{17} that was previously shown to have elevated DSBs within alpha heterochromatin in meiotic cells (Peng and Karpen 2009). Hypothesizing that heterochromatic crossover suppression is primarily chromatin-based, we expected to see a significantly greater number of crossovers in both heterochromatic regions of the pericentromere in this mutant compared with wild type and to both classes of meiotic mutants. Surprisingly, we saw no change from wild type in CE value or total crossover distribution patterns in proximal euchromatin or beta heterochromatin, suggesting that pericentromeric crossover suppression is not mediated by this H3K9 methyltransferase, despite it being a key component of pericentromeric heterochromatinization. It appears that heterochromatic crossovers are not suppressed during meiosis because they occur in heterochromatin and may be subject to steric hindrances, but by virtue of them being under the control of meiotic machinery.

Interestingly, we did recover one crossover between our most proximal SNPs in the *Su(var)3-9*^{06}/*Su(var)3-9*^{17} mutant. We believe this could be biologically relevant, as we observe complete suppression of crossovers in this region in wild-type flies. While this one crossover may be in unassembled beta heterochromatin, it is notable that *Su(var)3-9*^{06}/*Su(var)3-9*^{17} do not exhibit increased crossovers in beta heterochromatin. It is possible that this crossover was mitotic in origin. Among 3,393 progeny of *Su(var)3-9*^{06}/*Su(var)3-9*^{17} males, which do not have meiotic recombination, we recovered a single crossover, in beta heterochromatin (Supplementary Table 1 in Supplementary File 2). Mitotic crossovers in the male germline are extremely rare in wild-type males (McVey *et al.* 2007), so this may indicate a true increase in these mutants. We note that the elevated DSBs in female meiotic cells reported by Peng and Karpen (2009) may not behave like typical meiotic DSBs in terms of repair mechanisms and regulation.

Conclusions

Our study demonstrates that crossover control at the *Drosophila* pericentromere is multifaceted, and that a collaborative effort between diverse factors that include the SC, various recombination proteins, and even chromatin state may be necessary to establish or enforce the CE. We show that suppression of meiotic crossovers within heterochromatin appears to be influenced less, if at all, by the chromatin state and more by the meiotic machinery. Our data, in conjunction with studies from other labs, suggests that the mechanisms behind the CE may vary among chromosomes, providing fertile ground for future research on pericentromeric crossover suppression in *Drosophila* and other species.

Data availability

Drosophila stocks are available upon request. The authors confirm that all data necessary for confirming the conclusions of the article are present within the article, figures, table, and supplemental information. Illumina sequences for isogenized *OR+* and *net-cn* flies have been submitted to SRA under BioProject PRJNA1198609.

Supplemental material available at GENETICS online.

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Conflicts of interest

The funders did not play any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare that they have no conflicts of interest.

Literature cited

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, *et al.* 2000. The genome sequence of *Drosophila melanogaster*. Science. 287(5461): 2185–2195. doi:10.1126/science.287.5461.2185.

Baker BS, Carpenter AT. 1972. Genetic analysis of sex chromosomal meiotic mutants in *Drosophila melanogaster*. Genetics. 71(2): 255–286. doi:10.1093/genetics/71.2.255.

Beadle GW. 1932. A possible influence of the spindle fibre on crossing-over in Drosophila. Proc Natl Acad Sci U S A. 18(2):160–165. doi:10.1073/pnas.18.2.160.

Billmyre KK, Cahoon CK, Heenan GM, Wesley ER, Yu Z, Unruh JR, Takeo S, Hawley RS. 2019. X chromosome and autosomal recombination are differentially sensitive to disruptions in SC maintenance. Proc Natl Acad Sci U S A. 116(43):21641–21650. doi:10.1073/pnas.1910840116.

Blanton HL, Radford SJ, McMahan S, Kearney HM, Ibrahim JG, Sekelsky J. 2005. REC, Drosophila MCM8, drives formation of meiotic crossovers. PLoS Genet. 1(3):e40. doi:10.1371/journal.pgen.0010040.

Brand CL, Cattani MV, Kingan SB, Landeen EL, Presgraves DC. 2018. Molecular evolution at a meiosis dene mediates species differences in the rate and patterning of recombination. Curr Biol. 28(8):1289–1295.e1284. doi:10.1016/j.cub.2018.02.056.

Carpenter ATC. 1975. Electron microscopy of meiosis in *Drosophila melanogaster* females: II: the recombination nodule—a recombination-associated structure at pachytene? Proc Natl Acad Sci U S A. 72(8):3186–3189. doi:10.1073/pnas.72.8.3186.

Carpenter ATC. 1979. Recombination nodules and synaptonemal complex in recombination-defective females of *Drosophila melanogaster*. Chromosoma. 75(3):259–292. doi:10.1007/BF00293472.

Carpenter AT, Baker BS. 1982. On the control of the distribution of meiotic exchange in *Drosophila melanogaster*. Genetics. 101(1): 81–89. doi:10.1093/genetics/101.1.81.

Cléard F, Delattre M, Spierer P. 1997. SU(VAR)3-7, a Drosophila heterochromatin-associated protein and companion of HP1 in the genomic silencing of position-effect variegation. Embo J. 16(17):5280–5288. doi:10.1093/emboj/16.17.5280.

Collins KA, Unruh JR, Slaughter BD, Yu Z, Lake CM, Nielsen RJ, BoxKS, Miller DE, Blumenstiel JP, Perera AG, *et al.* 2014. Corolla is a novel protein that contributes to the architecture of the synaptonemal complex of Drosophila. Genetics. 198(1):219–228. doi:10.1534/genetics.114.165290.

Copenhaver GP, Nickel K, Kuromori T, Benito MI, Kaul S, Lin X, BevanM, Murphy G, Harris B, Parnell LD, *et al.* 1999. Genetic

definition and sequence analysis of Arabidopsis centromeres. Science. 286(5449):2468–2474. doi:10.1126/science.286.5449.2468.
Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, *et al.* 2021. Twelve years of SAMtools and BCFtools. GigaScience. 10(2): giab008. doi:10.1093/gigascience/giab008.
Delattre M, Spierer A, Tonka CH, Spierer P. 2000. The genomic silencing of position-effect variegation in *Drosophila melanogaster*: interaction between the heterochromatin-associated proteins Su(var)3-7 and HP1. J Cell Sci. 113(23):4253–4261. doi:10.1242/jcs.113.23.4253.
Fernandes JB, Naish M, Lian Q, Burns R, Tock AJ, Rabanal FA, Wlodzimierz P, Habring A, Nicholas RE, Weigel D, *et al.* 2024. Structural variation and DNA methylation shape the centromere-proximal meiotic crossover landscape in Arabidopsis. Genome Biol. 25(1):30. doi:10.1186/s13059-024-03163-4.
Fozard JA, Morgan C, Howard M. 2023. Coarsening dynamics can explain meiotic crossover patterning in both the presence and absence of the synaptonemal complex. eLife. 12:e79408. doi:10.7554/eLife.79408.
Gall JG, Cohen EH, Polan ML. 1971. Repetitive DNA sequences in Drosophila. Chromosoma. 33(3):319–344. doi:10.1007/BF00284948.
Garrisson E, Marth G. 2012. Haplotype-based variant detection from short-read sequencing. arXiv. 1207.3907. doi:10.48550/arXiv.1207.3907.
Ghaffari R, Cannon EK, Kanizay LB, Lawrence CJ, Dawe RK. 2013. Maize chromosomal knobs are located in gene-dense areas and suppress local recombination. Chromosoma. 122(1-2):67–75. doi:10.1007/s00412-012-0391-8.
Ghimire P, Motamedi M, Joh R. 2024. Mathematical model for the role of multiple pericentromeric repeats on heterochromatin assembly. PLoS Comput Biol. 20(4):e1012027. doi:10.1371/journal.pcbi.1012027.
Grell RF. 1978. Time of recombination in the *Drosophila melanogaster* oocyte: evidence from a temperature-sensitive recombination-deficient mutant. Proc Natl Acad Sci U S A. 75(7):3351–3354. doi:10.1073/pnas.75.7.3351.
Hartmann M, Kohl KP, Sekelsky J, Hatkevich T. 2019a. Meiotic MCM proteins promote and inhibit crossovers during meiotic recombination. Genetics. 212(2):461–468. doi:10.1534/genetics.119.302221.
Hartmann M, Umbanhowar J, Sekelsky J. 2019b. Centromere-proximal meiotic crossovers in *Drosophila melanogaster* are suppressed by both highly repetitive heterochromatin and proximity to the centromere. Genetics. 213(1):113–125. doi:10.1534/genetics.119.302509.
Hatkevich T, Kohl KP, McMahan S, Hartmann MA, Williams AM, Sekelsky J. 2017. Bloom syndrome helicase promotes meiotic crossover patterning and homolog disjunction. Curr Biol. 27(1): 96–102. doi:10.1016/j.cub.2016.10.055.
Hoskins RA, Carlson JW, Wan KH, Park S, Mendez I, Galle SE, Booth BW, Pfeiffer BD, George RA, Svirskas R, *et al.* 2015. The release 6 reference sequence of the *Drosophila melanogaster* genome. Genome Res. 25(3):445–458. doi:10.1101/gr.185579.114.
John B, King M. 1985. The inter-relationship between heterochromatin distribution and chiasma distribution. Genetica. 66(3): 183–194. doi:10.1007/BF00128039.
Koehler KE, Hawley RS, Sherman S, Hassold T. 1996. Recombination and nondisjunction in humans and flies. Hum Mol Genet. 5(Suppl. 1):1495–1504. doi:10.1093/hmg/5.Supplement_1.1495.
Kohl KP, Jones CD, Sekelsky J. 2012. Evolution of an MCM complex in flies that promotes meiotic crossovers by blocking BLM helicase. Science. 338(6112):1363–1365. doi:10.1126/science.1228190.
Lake CM, Hawley RS. 2012. The molecular control of meiotic chromosomal behavior: events in early meiotic prophase in Drosophila oocytes. Annu Rev Physiol. 74(1):425–451. doi:10.1146/annurev-physiol-020911-153342.
Lamb NE, Freeman SB, Savage-Austin A, Pettay D, Taft L, Hersey J, Gu Y, Shen J, Saker D, May KM, *et al.* 1996. Susceptible chiasmate configurations of chromosome 21 predispose to non–disjunction in both maternal meiosis I and meiosis II. Nat Genet. 14(4): 400–405. doi:10.1038/ng1296-400.
Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods. 9(4):357–359. doi:10.1038/nmeth.1923.
Libuda DE, Uzawa S, Meyer BJ, Villeneuve AM. 2013. Meiotic chromosome structures constrain and respond to designation of crossover sites. Nature. 502(7473):703–706. doi:10.1038/nature12577.
Mahtani MM, Willard HF. 1998. Physical and genetic mapping of the human X chromosome centromere: repression of recombination. Genome Res. 8(2):100–110. doi:10.1101/gr.8.2.100.
Martini E, Diaz RL, Hunter N, Keeney S. 2006. Crossover homeostasis in yeast meiosis. Cell. 126(2):285–295. doi:10.1016/j.cell.2006.05.044.
Mather K. 1939. Crossing over and heterochromatin in the X chromosome of *Drosophila melanogaster*. Genetics. 24(3):413–435. doi:10.1093/genetics/24.3.413.
Matsubayashi H, Yamamoto MT. 2003. REC, a new member of the MCM-related protein family, is required for meiotic recombination in Drosophila. Genes Genet Syst. 78(5):363–371. doi:10.1266/ggs.78.363.
McKim KS, Dahmus JB, Hawley RS. 1996. Cloning of the *Drosophila melanogaster* meiotic recombination gene *mei-218*: a genetic and molecular analysis of interval 15E. Genetics. 144(1):215–228. doi:10.1093/genetics/144.1.215.
McVey M, Andersen SL, Broze Y, Sekelsky J. 2007. Multiple Functions of Drosophila BLM Helicase in Maintenance of Genome Stability. Genetics. 176(4):1979–1992. doi:10.1534/genetics.106.070052.
Mehrotra S, Mckim KS. 2006. Temporal analysis of meiotic DNA double-strand break formation and repair in Drosophila females. PLoS Genet. 2(11):e200. doi:10.1371/journal.pgen.0020200.
Miklos GL, Cotsell JN. 1990. Chromosome structure at interfaces between major chromatin types: alpha- and beta heterochromatin. Bioessays. 12(1):1–6. doi:10.1002/bies.950120102.
Morgan C, Fozard JA, Hartley M, Henderson IR, Bomblies K, Howard M. 2021. Diffusion-mediated HEI10 coarsening can explain meiotic crossover positioning in Arabidopsis. Nat Commun. 12(1):4674. doi:10.1038/s41467-021-24827-w.
Nambiar M, Smith GR. 2016. Repression of harmful meiotic recombination in centromeric regions. Semin Cell Dev Biol. 54: 188–197. doi:10.1016/j.semcdb.2016.01.042.
Nambiar M, Smith GR. 2018. Pericentromere-specific cohesin complex prevents meiotic pericentric DNA double-strand breaks and lethal crossovers. Mol Cell. 71(4):540–553.e544. doi:10.1016/j.molcel.2018.06.035.
Oliver TR, Tinker SW, Allen EG, Hollis N, Locke AE, Bean LJH, Chowdhury R, Begum F, Marazita M, Cheung V, *et al.* 2012. Altered patterns of multiple recombinant events are associated with nondisjunction of chromosome 21. Hum Genet. 131(7): 1039–1046. doi:10.1007/s00439-011-1121-7.
Owen ARG. 1950. The theory of genetical recombination. In: Demerec M, editors. Advances in Genetics. Cold Spring Harbor, NY: Academic Press. p. 117–157.
Öztürk-Çolak A, Marygold SJ, Antonazzo G, Attrill H, Goutte-Gattat D, Jenkins VK, Matthewes BB, Millburn G, do Santos G, Tabone CJ; FlyBase Constortium. 2024. FlyBase: updates to the Drosophila genes and genomes database. Genetics. 227(1):iyad211. doi:10.1093/genetics/iyad211.

Page SL, Hawley RS. 2001. C(3)G encodes a Drosophila synaptonemal complex protein. Genes Dev. 15(23):3130–3143. doi:10.1101/gad.935001.

Pazhayam NM, Frazier LK, Sekelsky J. 2024. Centromere-proximal suppression of meiotic crossovers in Drosophila is robust to changes in centromere number and repetitive DNA content. Genetics. 226(3):iyad216. doi:10.1093/genetics/iyad216.

Pazhayam NM, Turcotte CA, Sekelsky J. 2021. Meiotic crossover patterning. Front Cell Dev Biol. 9:681123. doi:10.3389/fcell.2021.681123.

Peng JC, Karpen GH. 2007. H3k9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. Nat Cell Biol. 9(1):25–35. doi:10.1038/ncb1514.

Peng JC, Karpen GH. 2009. Heterochromatic genome stability requires regulators of histone H3 K9 methylation. PLoS Genet. 5(3):e1000435. doi:10.1371/journal.pgen.1000435.

Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. 2011. Integrative genomics viewer. Nat Biotechnol. 29(1):24–26. doi:10.1038/nbt.1754.

Rog O, Köhler S, Dernburg AF. 2017. The synaptonemal complex has liquid crystalline properties and spatially regulates meiotic recombination factors. Elife. 6:e21455. doi:10.7554/eLife.21455.

Sandler L, Szauter P. 1978. The effect of recombination-defective meiotic mutants on fourth-chromosome crossing over in *Drosophila melanogaster*. Genetics. 90(4):699–712. doi:10.1093/genetics/90.4.699.

Schotta G, Ebert A, Krauss V, Fischer A, Hoffmann J, Rea S, Jenuwein T, Dorn R, Reuter G. 2002. Central role of Drosophila SU(VAR)3-9 in histone H3-K9 methylation and heterochromatic gene silencing. EMBO J. 21(5):1121–1131. doi:10.1093/emboj/21.5.1121.

Simon L, Voisin M, Tatout C, Probst AV. 2015. Structure and function of centromeric and pericentromeric heterochromatin in Arabidopsis thaliana. Front Plant Sci. 6:1049. doi:10.3389/fpls.2015.01049.

Slatis HM. 1955. A reconsideration of the brown-dominant position effect. Genetics. 40(2):246–251. doi:10.1093/genetics/40.2.246.

Storlazzi A, Xu L, Schwacha A, Kleckner N. 1996. Synaptonemal complex (SC) component Zip1 plays a role in meiotic recombination independent of SC polymerization along the chromosomes. Proc Natl Acad Sci U S A. 93(17):9043–9048. doi:10.1073/pnas.93.17.9043.

Sturtevant AH. 1913. The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association. J Exp Zool. 14(1):43–59. doi:10.1002/jez.1400140104.

Stutzman AV, Hill CA, Armstrong RL, Gohil R, Duronio RJ, Dowen JM, McKay DJ. 2024. Heterochromatic 3D genome organization is directed by HP1a- and H3K9-dependent and independent mechanisms. Mol Cell. 84:2017–2035.e2016. doi:10.1016/j.molcel.2024.05.002.

Sym M, Roeder GS. 1994. Crossover interference is abolished in the absence of a synaptonemal complex protein. Cell. 79(2):283–292. doi:10.1016/0092-8674(94)90197-X.

Szauter P. 1984. An analysis of regional constraints on exchange in *Drosophila melanogaster* using recombination-defective meiotic mutants. Genetics. 106(1):45–71. doi:10.1093/genetics/106.1.45.

Vincenten N, Kuhl L-M, Lam I, Oke A, Kerr AR, Hochwagen A, Fung J, Keeney S, Vader G, Marston AL. 2015. The kinetochore prevents centromere-proximal crossover recombination during meiosis. Elife. 4:e10850. doi:10.7554/eLife.10850.

Voelkel-Meiman K, Cheng SY, Morehouse SJ, MacQueen AJ. 2016. Synaptonemal complex proteins of budding yeast define reciprocal roles in MutSγ-mediated crossover formation. Genetics. 203(3):1091–1103. doi:10.1534/genetics.115.182923.

Voelkel-Meiman K, Johnston C, Thappeta Y, Subramanian VV, Hochwagen A, MacQueen AJ. 2015. Separable crossover-promoting and crossover-constraining aspects of zip1 activity during budding yeast meiosis. PLoS Genet. 11(6):e1005335. doi:10.1371/journal.pgen.1005335.

von Diezmann L, Bristow C, Rog O. 2024. Diffusion within the synaptonemal complex can account for signal transduction along meiotic chromosomes. Mol Biol Cell. 35(12):ar148. doi:10.1091/mbc.E24-05-0225.

Wang K, Wang C, Liu Q, Liu W, Fu Y. 2015. Increasing the genetic recombination frequency by partial loss of function of the synaptonemal complex in rice. Mol Plant. 8(8):1295–1298. doi:10.1016/j.molp.2015.04.011.

Westphal T, Reuter G. 2002. Recombinogenic effects of suppressors of position-effect variegation in Drosophila. Genetics. 160(2):609–621. doi:10.1093/genetics/160.2.609.

Wu J, Mizuno H, Hayashi-Tsugane M, Ito Y, Chiden Y, Fujisawa M, Katagiri S, Saji S, Yoshiki S, Karasawa W, *et al.* 2003. Physical maps and recombination frequency of six rice chromosomes. Plant J. 36(5):720–730. doi:10.1046/j.1365-313X.2003.01903.x.

Xue M, Wang J, Jiang L, Wang M, Wolfe S, Pawlowski WP, Wang Y, He Y. 2018. The number of meiotic double-strand breaks influences crossover distribution in Arabidopsis. Plant Cell. 30(10):2628–2638. doi:10.1105/tpc.18.00531.

Yamamoto M, Miklos GL. 1978. Genetic studies on heterochromatin in r*Drosophila melanogaster* and their implications for the functions of satellite DNA. Chromosoma. 66(1):71–98. doi:10.1007/BF00285817.

Zhang L, Stauffer W, Zwicker D, Dernburg AF. 2021. Crossover patterning through kinase-regulated condensation and coarsening of recombination nodules. bioRxiv 457865. doi:10.1101/2021.08.26.457865, preprint: not peer reviewed.

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